REMARKS

Rejections Under 35 USC §112, 1st Paragraph

Claims 1, 3-6 and 8-9 stand rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. The Examiner states that the specification, while being enabling for a method of using peritoneal macrophages to induce systemic T cell tolerance, does not reasonably provide enablement for inducing systemic tolerance using antigen presenting cells other than fas-negative peritoneal macrophages. This rejection is respectfully traversed.

The Examiner contends that the term "antigen presenting cell" is broad and encompasses any cell type that can present antigen in the context of MHC Class I or Class II to a T cell. While the Examiner acknowledges that Class II MHC is only expressed on a subset of cells known as professional antigen presenting cells, the Examiner contends that Class I MHC is ubiquitously expressed in all types of normal cells. Accordingly, the Examiner interprets antigen presenting cells as including numerous Class I MHC-expressing cell

types such as fibroblasts, epithelial cells, lymphomas, hepatocytes and myocytes. Applicant respectfully disagrees.

Applicant submits that one of ordinary skill in the art would not interpret antigen-presenting cells as including all Class I MHC-expressing cells. Although all the cells expressing either class I or class II molecules can present peptides to T cells, not all of them are designated as antigen presenting cells. Cells that display peptides associated with class I MHC molecules to CD8T cells are referred to as target cells whereas cells that display peptides associated with class II MHC molecules to CD4+ T helper cells are called antigen-presenting cells. The antigen-presenting cells are further classified as professional and non-professional antigen-presenting cells. Professional antigen presenting cells are dendritic cells, macrophages and B lymphocytes that express MHC class II molecules and co-stimulatory molecules either before or after they are activated. In contrast, non-professional antigen cells which comprise of fibroblasts, glial cells, pancreatic beta cells, thymic epithelial cells, thyroid epithelial cells and vascular endothelial cells are cells that are induced to express class II MHC molecules or a

costimulatory signal (see attached excerpt from Immunology, 3rd edition, Janis Kuby, Page 253, line 1-31, Table 10-1).

The present invention is drawn to a method of using genetically modified Fas ligand-expressing antigen presenting cells to induce apoptosis of Fas-positive antigen-specific T cells, thereby inducing systemic tolerance to the antigen. The instant specification has provided data on using macrophages such as peritoneal macrophages to induce systemic tolerance. These peritoneal macrophages are similar to the macrophages and definitely different from fibroblasts and the other cells specified by the Examiner. Applicant submits that the claimed method is enabling in view of the present specification and the knowledge and skill in the art. Further, the claimed method has also been successfully used with dendritic cells. It has been reported that monocyte-derived human dendritic cells transduced by the adenoviral vectors disclosed herein induced apoptosis in Fas+ Jurkat T cells in coculture studies (Hover et al. (2003), *J. Immunol.* 170:5406-5413). Subsequent studies indicated that these genetically modified Fas ligand-expressing human dendritic cells induced apoptosis in activated but not resting

$CD4^+$ and $CD8^+$ T cells (Hover et al. (2004), *Immunobiology* 208:463-475).

Given the elimination of activated T cells by these modified dendritic cells, it is logical and reasonable to conclude that T cell-mediated immune response would be reduced *in vivo* and tolerance would be accomplished. Taken together, disclosure in the specification and published reports in the art indicate that the claimed method of inducing tolerance can be and has been successfully used with macrophages and dendritic cells, which are cells ordinarily regarded as professional antigen presenting cells in the art.

The Examiner further contends that there is a lack of predictability in the art. The Examiner contends that cited references established that at the time of filing, a controversy existed in the art as to the ability of Fas ligand to inhibit graft rejection or induce T cell apoptosis. A review by Restifo discusses the fact that although the idea that Fas ligand expression could grant immune privilege status rapidly gained popularity, substantial evidence to the contrary exists in the literature (Restifo (2000), Nat. Med. 6:493-495). Numerous papers cited by Restifo document the

fact that expression of recombinant Fas ligand by many different cell types results in inflammatory responses *in vivo* rather than tolerance. Kang et al. (1998) has also been reported that islet cells, fibroblasts, epithelial cells, and various tumor cell lines genetically modified to express Fas ligand are rapidly rejected *in vivo* as a result of profound inflammatory response. Thus, the Examiner concludes that it is unpredictable that Fas ligand expression in any antigen presenting cells would result in immunosupression rather than inflammation *in vivo*. Applicant respectfully disagrees.

Applicant submits that induction of inflammation by Fas ligand-expressing islet cells, fibroblasts, epithelial cells, and various tumor cell lines is not relevant to the instant invention. As discussed above, one of ordinary skill in the art would not ordinarily regard islet cells, fibroblasts, epithelial cells, and various tumor cell lines as antigen presenting cells taught in the present invention. Further, it is known in the art that interaction of Fas-FasL promotes apoptosis. Apoptosis and necrosis are two very different types of cell death. Necrosis is associated with cell death arising from injury where the injured cells swell and bursts, releasing intracellular contents, which are cytotoxic to other cells in the tissue and results

in an inflammatory response. In distinct contrast, apoptosis occurs without inducing localized inflammatory response. In apoptosis, the cell undergoes morphological changes such as decrease in volume, modification of the cytoskeleton resulting in membrane blebbing, condensation of chromatin and degradation of DNA into oligonucleosomal fragments. Following these changes, the apoptotic cell sheds tiny membrane-bound apoptotic bodies containing intact organelles, which are phagocytosed by macrophages, thus preventing the intracellular contents from being released into the surrounding. (Immunology, 3rd Edition, Janis Kuby, page 53, col. II, last para-page 54, col. I, Table 3-2). Additionally, the instant specification and published reports have consistently demonstrated that Fas ligand-expressing antigen presenting cells such as macrophages or dendritic cells are capable of inducing tolerance *in vivo*. Thus, there is no unpredictability with regard to induction of tolerance by Fas ligand-expressing antigen presenting cells that are well-recognized in the art as macrophages or dendritic cells.

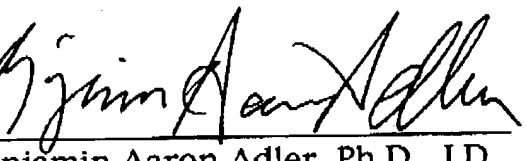
In view of the above remarks, Applicant submits that the claimed method of using antigen presenting cells to induce systemic T cell tolerance is commensurate with the scope of disclosure

provided in the specification and general knowledge in the art, and the claimed method is fully enabled. Accordingly, Applicant respectfully requests that the rejection of claims 1, 3-6 and 8-9 under 35 U.S.C. §112, first paragraph, be withdrawn.

This is intended to be a complete response to the Final Office Action mailed September 23, 2004. If any issues remain outstanding, the examiner is respectfully requested to telephone the undersigned attorney of record for immediate resolution.

Respectfully submitted,

Date: Feb 22, 2005



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cells. However, by convention, cells that display peptides associated with class I MHC molecules to $CD8^+$ T_C cells are referred to as **target cells**; cells that display peptides associated with class II MHC molecules to $CD4^+$ T_H cells are called **antigen-presenting cells** (APCs). This convention is followed throughout this text.

A variety of cells can function as antigen-presenting cells. The distinguishing feature of these cells is their ability to express class II MHC molecules and to deliver a co-stimulatory signal. Three cell types are classified as **professional** antigen-presenting cells: dendritic cells, macrophages, and B lymphocytes. These cells differ in their mechanisms of antigen uptake, in whether they constitutively express class II MHC molecules, and in their co-stimulatory activity:

- Dendritic cells are the most effective of the antigen-presenting cells. Because these cells constitutively express a high level of class II MHC molecules and of co-stimulatory activity, they can activate naive T_H cells.
- Macrophages must be activated by phagocytosing microorganisms before they express class II MHC molecules or the co-stimulatory B7 membrane molecule.
- B cells constitutively express class II MHC molecules but must be activated before they express the co-stimulatory B7 molecule.

Several other cell types, classified as **nonprofessional** antigen-presenting cells, can be induced to express class II MHC molecules or a co-stimulatory signal (Table 10-1). Many of these cells function in antigen presentation only for short periods of time during a sustained inflammatory response.

Because nearly all nucleated cells express class I MHC molecules, virtually any nucleated cell is able to function as a target cell presenting endogenous antigens to T_C cells. Most often target cells are cells that have been infected by a virus or some other intracellular microorganism. However, target cells can also be altered self-cells such as cancer cells, aging body cells, or allogeneic cells from a graft.

TABLE 10-1

ANTIGEN-PRESENTING CELLS

Professional antigen-presenting cells
Dendritic cells (several types)
Macrophages
B cells
Nonprofessional antigen-presenting cells
Fibroblasts (skin)
Glial cells (brain)
Pancreatic beta cells
Thymic epithelial cells
Thyroid epithelial cells
Vascular endothelial cells

EVIDENCE FOR TWO PROCESSING AND PRESENTATION PATHWAYS

Intracellular (endogenous) and extracellular (exogenous) antigens present different challenges to the immune system. Extracellular antigens are eliminated by secreted antibody, whereas intracellular antigens are most effectively eliminated by cytotoxic T lymphocytes (CTLs). To mediate these responses, the immune system uses two different antigen-presenting pathways: endogenous antigens are processed in the **cytosolic pathway** and presented on the membrane with class I MHC molecules; exogenous antigens are processed in the **endocytic pathway** and presented on the membrane with class II MHC molecules (Figure 10-4).

Early evidence suggesting that class I and class II MHC molecules present antigenic peptides derived from different processing pathways was obtained from experiments with two clones of T_C cells specific for influenza virus. One clone was a typical $CD8^+$, class I–restricted T_C cell, but the other was an atypical $CD4^+$, class II–restricted T_C cell. As discussed in Chapter 3, the association between T-cell function and MHC restriction is not absolute. Indeed, an increasing number of reports have described cross-functional T-cell lines—that is, $CD4^+$, class II–restricted T_C clones, and $CD8^+$, class I–restricted T_H clones. L. A. Morrison and T. J. Braciale analyzed two T_C cell lines: one a typical T_C line that recognized influenza hemagglutinin (HA) associated with a class I MHC molecule and the other an atypical T_C line that recognized influenza HA associated with a class II MHC molecule. These researchers sought to determine whether antigen is processed along different pathways for association with class I or class II MHC molecules. In one set of experiments, target cells that expressed both class I and class II MHC molecules were incubated with infectious influenza virus or with UV-inactivated influenza virus. (The inactivated virus retained its antigenic properties but was no longer capable of replicating

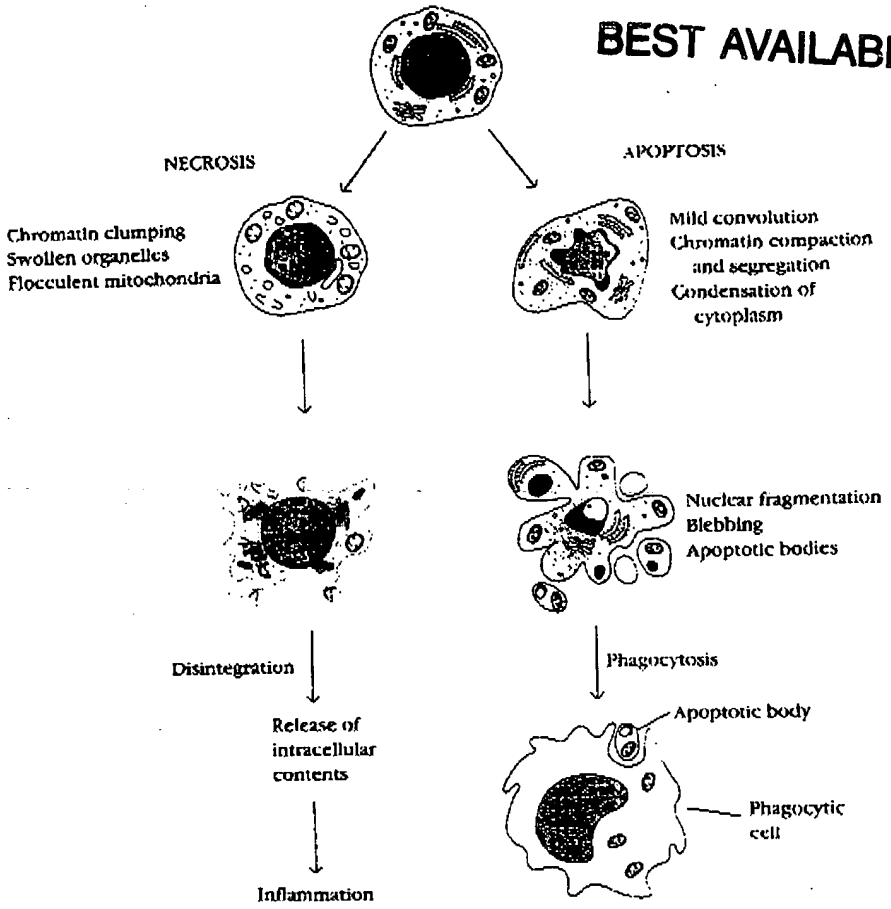

FIGURE 3-4

Comparison of morphologic changes that occur in apoptosis and necrosis. Apoptosis, which is associated with the programmed cell death of hematopoietic cells, does not induce a localized inflammatory response. In contrast, necrosis, the process leading to death of injured cells, results in release of the intracellular contents, which induce a localized inflammatory response.

With fewer receptors on its membrane, the cell becomes progressively less responsive to the CSF, and proliferation of the lineage slows down. This down-modulation of CSF-receptor expression can even be induced by the binding of unrelated CSFs to their receptors. For example, when GM-CSF binds to its receptor, it induces the cell to down-modulate the expression of G-CSF and M-CSF receptors as well. This down-modulation of G-CSF and M-CSF receptors causes the lineages bearing these receptors to become less responsive to these CSFs.

Hematopoiesis can also be regulated by degradation of a CSF following its binding to a receptor. Experiments suggest that binding of M-CSF to its receptor results in degradation of the cytokine. As monocyte numbers increase, there is a corresponding increase in M-CSF receptors, leading to increased M-CSF degradation. Thus the M-CSF concentration falls as cell numbers increase, thereby slowing further proliferation and differentiation of this lineage as long as the number of monocytes remains high.

Programmed Cell Death

In order for steady-state levels of the various hematopoietic cells to be maintained, cell division and differentiation in each of the lineages is balanced by a process called **programmed cell death**. Cells undergoing programmed cell death often exhibit distinctive morphologic changes, collectively referred to as **apoptosis** (Figure 3-4). These changes include a pronounced decrease in cell volume, modification of the cytoskeleton resulting in pronounced membrane blebbing, a condensation of the

chromatin, and degradation of the DNA into oligonucleosomal fragments. Following these morphologic changes, an apoptotic cell sheds tiny membrane-bound apoptotic bodies containing intact organelles. Macrophages quickly phagocytose apoptotic bodies, ensuring that their intracellular contents, including proteolytic and other lytic enzymes, cationic proteins, and oxidizing molecules are not released into the surrounding tissue. In this way apoptosis occurs without inducing a localized inflammatory response. Apoptosis differs markedly from **necrosis**, the changes associated with cell death arising from injury. In necrosis the injured cell swells and bursts, releasing its intracellular contents, which are cytotoxic to other cells in the tissue; as a result, an inflammatory response develops.

Each of the cells produced by hematopoiesis has a characteristic life span and then dies by programmed cell death. In the adult human, for example, there are about 5×10^{10} neutrophils in the circulation. These cells have a life span of only 1 day and then die by programmed cell death. This death, coupled with constant neutrophil production, maintains steady-state levels of these cells. If programmed cell death fails to occur, a leukemic state may develop. Programmed cell death also plays a role in maintaining proper levels of hematopoietic progenitor cells. For example, when colony-stimulating factors are removed, progenitor cells undergo programmed cell death.

The expression of several genes has been associated with the regulation of apoptosis in hematopoietic cell lineages (Table 3-2). Some of these gene products induce apoptosis, whereas other gene products inhibit apoptosis. The *bcl-2* (B-cell lymphoma 2) gene, for example, encodes a protein product that inhibits apoptosis. This gene was originally identified at the breakpoint of a chromosomal translocation in a human B-cell lymphoma. This translocation moved the *bcl-2* gene into the immunoglobulin heavy-chain locus, resulting in transcriptional activation of the *bcl-2* gene and overproduction of the encoded Bcl-2 protein by the lymphoma cells. The resulting high levels of Bcl-2 are thought to contribute to transformation of lymphoid cells into cancerous lymphoma cells by inhibiting the normal signals that would induce apoptotic cell death.

Bcl-2 levels have been found to play an important role in regulating the normal life span of various hematopoietic cell lineages, including lymphocytes. A normal adult has about 5 L of blood with about 2000 lymphocytes/mm^3, for a total of about 10 billion lymphocytes. During acute infection the lymphocyte count increases by 4- to 15-fold, giving a total lymphocyte count of 40–150 billion. Because the immune system cannot sustain such a massive increase in cell numbers for an extended period, the system needs a means to eliminate unneeded activated lymphocytes once the antigenic threat has passed. Activated lymphocytes have been found to express lower levels of Bcl-2 and therefore are more susceptible to apoptotic death than naive lymphocytes or memory cells. If the lymphocytes continue to be activated by antigen, then the signals received during activation bypass the apoptotic signal. As antigen levels subside, so does activation of the lymphocytes and they begin to die by apoptosis (Figure 3-5).

TABLE 3-2

GENES THAT REGULATE APOPTOSIS

GENE	FUNCTION	ROLE IN PROGRAMMED CELL DEATH
bcl-2	Prevents apoptosis	Prevents
bax	Opposes *bcl-2*	Promotes
bcl-X^L (long)	Prevents apotosis	Prevents
bcl-X^S (short)	Opposes *bcl-X*^{L}	Promotes
ICE *	Protease	Promotes
fas/apo-1	Promotes apoptosis	Promotes

* Gene encoding the interleukin 1β–converting enzyme.

Regulatory Abnormalities and Leukemia

Abnormalities in the expression of hematopoietic cytokines or their receptors may result in some leukemias. Colony-stimulating factors are secreted by a limited number of cells, including activated T lymphocytes, macrophages, endothelial cells, and bone-marrow stromal cells. As mentioned above, each factor induces the proliferation and differentiation of only those hematopoietic stem cells and progenitor cells that bear its receptor. Expression of receptors for a particular growth factor appears to be linked to cellular differentiation following proliferation induced by earlier-acting growth factors. A defect in regulation of expression of either the growth factor or its receptor could lead to unregulated cellular proliferation.

For example, failure to down-modulate receptor expression following GM-CSF activation may lead to a leukemic state. The binding of GM-CSF induces down-modulation of both G-CSF and M-CSF receptors on normal hematopoietic cells but not on leukemic cells (Figure 3-6a, b). This failure of GM-CSF to down-modulate the G-CSF or M-CSF receptors on leukemic cells may allow leukemic cells to respond to low levels of